



# Evaluation of the Long-Term Treatment Performance of a Submerged Anaerobic Membrane Bioreactor for Phenolic Wastewater Treatment

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**Abstract** This study evaluates the long-term performance of a submerged anaerobic membrane bioreactor (SAnMBR) equipped with a hollow-fiber membrane for the treatment of synthetic industrial wastewater containing 2,4-dichlorophenol (2,4-DCP), a toxic and persistent phenolic compound. Operated continuously for 189 days at  $36 \pm 1$  °C, the system demonstrated high and stable removal efficiencies, achieving a maximum removal of 99.63% for chemical oxygen demand (COD) and 99.6% for 2,4-DCP. Following the introduction of 2,4-DCP from day 118, the bioreactor exhibited a temporary decline in removal efficiency due to microbial stress, but quickly recovered, confirming strong microbial acclimation and process robustness. The average COD and 2,4-DCP removal rates were both 91.9%. Turbidity decreased by 63.98%, while total suspended solids

(TSS) and total dissolved solids (TDS) removal efficiencies reached 95% and 94%, respectively. The hollow-fiber membrane module enhanced solid–liquid separation, contributed to fouling resistance, and supported stable trans-membrane pressure (TMP) over the long term. The bioreactor effectively treated varying organic loading rates (0.125–0.798 kg COD/m<sup>3</sup>·d), showcasing operational flexibility. Compared to conventional anaerobic systems, the SAnMBR presented superior performance without the need for auxiliary carbon sources, highlighting its suitability for high-strength phenolic wastewater.

**Keywords** Submerged membrane bioreactors · 2,4-dichlorophenol wastewater · Hollow fiber membrane · Industrial wastewater treatment

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## 1 Introduction

The rapid growth of population and industrial activities has led to increased water consumption and the discharge of complex pollutants into the environment. Phenolic compounds, particularly 2,4-dichlorophenol (2,4-DCP), are commonly found in the effluents of gas production, coke oven operations, pharmaceuticals, petroleum refining, and plastics industries (Chunyan et al., 2023). Phenol concentrations in high-strength industrial wastewater can reach up to 5000 mg/L (Wu et al., 2020). These compounds are known for their persistence and hazardous effects, including endocrine disruption, reproductive toxicity, mutagenicity, and carcinogenicity (Zheng et al., 2019). When present in chlorinated drinking water, phenols can form chlorophenols, leading to unpleasant taste, odor, and serious health risks (Kim et al., 2023).

Conventional treatment technologies, such as adsorption, chemical oxidation, and extraction, are frequently employed for phenol removal but often transfer rather than degrade pollutants, potentially generating more harmful byproducts (Pandian et al., 2021; Wu et al., 2020). Biological treatment offers a safer, more sustainable alternative. However, traditional aerobic systems are often ineffective for phenol-rich wastewater due to microbial inhibition, particularly in sectors like oil processing (Sathya et al., 2023).

Anaerobic digestion is a promising method for phenolic wastewater treatment, enabling organic matter degradation and methane production without the need for oxygen (Wang et al., 2019). Studies using anaerobic sequencing batch reactors and up-flow anaerobic sludge blanket (UASB) systems report promising results (Franchi et al., 2020). However, these systems are often limited by biomass washout and reduced performance under shock loading.

Anaerobic membrane bioreactors (AnMBRs) offer a promising solution by integrating biological treatment with membrane filtration. This design decouples hydraulic retention time from sludge retention time, enhances microbial retention, and increases resilience to variable loading (Wu et al., 2023). Among AnMBRs, submerged configurations (SAnMBRs) reduce energy consumption and membrane fouling due to internal gas sparging (Gao et al., 2016). Hollow-fiber membranes provide high surface area and biofilm support (Shen et al., 2018; Tian et al., 2020).

Despite these benefits, studies on SAnMBRs treating chlorinated phenolic compounds like 2,4-DCP remain limited.

There is a lack of comprehensive, long-term studies evaluating the stability, removal efficiency, and resilience of SAnMBRs treating high-strength phenolic wastewater, especially under variable organic loading and toxic conditions using hollow-fiber membranes. A submerged anaerobic membrane bioreactor equipped with hollow-fiber membranes can maintain high and stable removal of COD and 2,4-DCP over extended periods, even under varying load and inhibitory stress. This study aims to bridge this gap by investigating the long-term performance of a pilot-scale SAnMBR treating synthetic wastewater containing 2,4-DCP. The work provides insights into the reactor's operational stability, pollutant removal efficiency, and membrane behaviour under increasing influent concentrations and organic loads.

## 2 Methods and Materials

### 2.1 Reactor Setup and Experimental Procedure

This study was conducted using a pilot-scale anaerobic submerged membrane bioreactor (AnSMBR) over a continuous operational period of 189 days. A custom-built Plexiglas reactor was designed for this purpose, comprising two functional zones: a lower anaerobic digestion chamber responsible for the biological degradation of organic matter, and an upper filtration zone housing hollow fiber ultrafiltration membranes. The reactor had an effective working volume of 8 L. The influent organic loading rate (OLR) was varied throughout the experiment, ranging from 0.125 to 0.798 kg COD/m<sup>3</sup>·day, to evaluate the system's performance under different loading conditions. On day 118, the xenobiotic compound 2,4-dichlorophenol (2,4-DCP) was introduced into the feed to assess its effect on treatment performance.

The membrane system consisted of hollow fiber ultrafiltration modules with a nominal pore size of 0.045 µm and an effective surface area of 0.93 m<sup>2</sup>. Filtration was performed under suction using a peristaltic pump, and the permeate was collected in a sealed storage container. To minimize membrane fouling, the system incorporated periodic permeate backflushing and relaxation intervals. The filtration

cycle consisted of 7.5 min of filtration, followed by a 5-s relaxation, 0.5 min of backflush, and another 5-s relaxation, resulting in a net filtration time of 91.91% per cycle.

Prior to operation, seed sludge was obtained and thoroughly washed multiple times with tap water to eliminate residual impurities. The cleaned sludge was then analyzed in triplicate to determine its physico-chemical characteristics, and the average values are presented in Table 1. After characterization, the prepared sludge was introduced into the reactor, which was subsequently brought into operation.

A schematic of the AnSMBR system is shown in Fig. 1. The reactor consisted of a lower anaerobic digestion zone and an upper filtration section containing two hollow fiber ultrafiltration membranes. Raw wastewater was fed using peristaltic pumps, and permeate was withdrawn under suction and directed to

a storage tank. Backwashing with stored permeate and air scouring via compressors were employed to reduce membrane fouling. Effluent passed through a condensate trap before discharge, while a safety bottle was installed to prevent contamination during backflow.

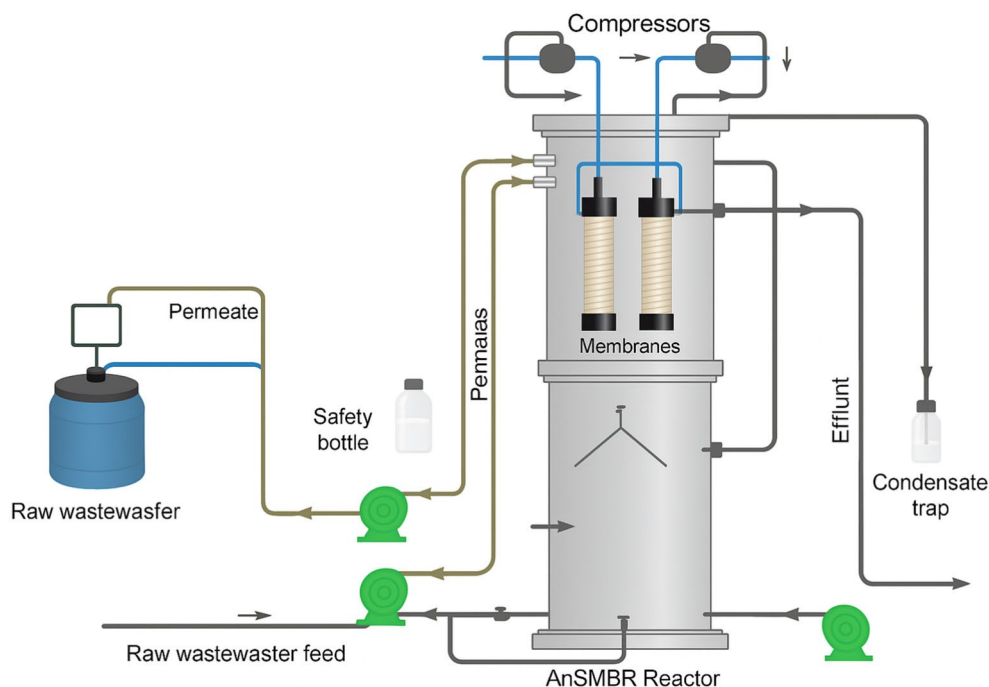
## 2.2 Wastewater Composition

A synthetic wastewater solution was prepared daily to serve as the substrate for the submerged anaerobic membrane bioreactor (SAnMBR). Glucose was used as the primary carbon source, supplemented with a comprehensive mix of macro- and micronutrients to support microbial growth and maintain reactor performance. These constituents were selected to mimic the nutrient profile commonly found in industrial effluents containing phenolic compounds. Table 2 summarizes the composition of the synthetic wastewater used in this study, expressed per gram of COD. This formulation ensured balanced nutrient availability and trace metal support for anaerobic biodegradation pathways.

The membrane module was submerged within the main reactor (Fig. 2) chamber. Hollow fiber

**Table 1** Characteristics of sludge used after washing for bio-reactor seeding

| Volume used for seeding (ml) | VSS (mg/lit) | TTS (mg/lit) |
|------------------------------|--------------|--------------|
| 2000                         | 12937        | 21082        |



**Fig. 1** Schematic flow diagram of the SAnMBR system

**Table 2** Composition of synthetic wastewater used in the SAnMBR system

| Component                       | Chemical formula                                                     | Concentration (mg/g COD) |
|---------------------------------|----------------------------------------------------------------------|--------------------------|
| Carbon source                   | Glucose                                                              | Variable (to match COD)  |
| Ammonium chloride               | NH <sub>4</sub> Cl                                                   | 76.45                    |
| Potassium dihydrogen phosphate  | KH <sub>2</sub> PO <sub>4</sub>                                      | 10.00                    |
| Dipotassium hydrogen phosphate  | K <sub>2</sub> HPO <sub>4</sub>                                      | 25.32                    |
| Ferric chloride                 | FeCl <sub>3</sub>                                                    | 1.021                    |
| Calcium chloride dihydrate      | CaCl <sub>2</sub> ·2H <sub>2</sub> O                                 | 2.06                     |
| Magnesium sulfate heptahydrate  | MgSO <sub>4</sub> ·7H <sub>2</sub> O                                 | 2.14                     |
| Manganese sulfate monohydrate   | MnSO <sub>4</sub> ·H <sub>2</sub> O                                  | 0.355                    |
| Cobalt(II) chloride hexahydrate | CoCl <sub>2</sub> ·6H <sub>2</sub> O                                 | 0.092                    |
| Nickel sulfate hexahydrate      | NiSO <sub>4</sub> ·6H <sub>2</sub> O                                 | 0.0763                   |
| Zinc sulfate                    | ZnSO <sub>4</sub>                                                    | 0.1055                   |
| Ammonium molybdate tetrahydrate | (NH <sub>4</sub> )Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O | 0.4198                   |
| Copper(II) sulfate pentahydrate | CuSO <sub>4</sub> ·5H <sub>2</sub> O                                 | 0.0321                   |
| Boric acid                      | H <sub>3</sub> BO <sub>3</sub>                                       | 0.020                    |

**Fig. 2** Illustration of hollow fiber membranes

membranes made of polypropylene (Hydrol, UK), were employed, providing a total membrane surface area of 0.1 m<sup>2</sup>. The raw wastewater inlet and effluent outlet pipes were installed at the top of the reactor. Two parallel peristaltic pumps (Shiva Amvaj, Iran) were used to regulate the influent and effluent flow rates, controlled by a digital multi-timer (Shiva Amvaj, Isfahan, Iran). To maintain a stable operating temperature of 36 ± 1 °C, the

system was equipped with temperature-controlled liquid baths containing vertically mounted heaters on the lateral walls. A digital thermometer was used for continuous temperature monitoring. An aquarium pump was used to circulate water in the bath, ensuring uniform temperature distribution. To minimize water loss due to evaporation and prevent salt deposition on the bath walls, distilled water was used and the bath surface was covered with plastic film.

### 2.3 Operating Conditions

The reactor was operated continuously for 189 days. The system was seeded with 2000 mL of digested sludge obtained from the South Tehran wastewater treatment plant. Prior to seeding, the sludge was washed several times with tap water to remove impurities and was characterized for its volatile suspended solids (VSS) and total suspended solids (TSS), which were measured at 12,937 mg/L and 21,082 mg/L, respectively.

The organic loading rate (OLR) was gradually increased from 0.125 to 0.798 kg COD/m<sup>3</sup>·day. The injection of 2,4-DCP began on day 118, with its concentration incrementally raised from 5 mg/L to 300 mg/L. Reactor performance was evaluated by monitoring the removal efficiencies of COD and 2,4-DCP under the varying loading conditions.

## 2.4 Analytical Methods

In this study, several physicochemical parameters were measured, including temperature, chemical oxygen demand (COD), pH, turbidity, total suspended and soluble solids (TSS), volatile suspended solids (VSS), 2,4-DCP concentration, electrical conductivity (EC) and animosity parameters were measured. COD and phenol were analysed using the closed reflux colorimetric method (APHA, 5220D) and direct photometric method (APHA, 5530D) (Miner, 2006), respectively, with a spectrophotometer (DR 6000, Hach, Germany). Turbidity was analysed using the Nephelometric Method (Miner, 2006) (Miner, 2006) and measured with a 2100 AN spectrophotometer (Hach, Germany). The pH was regulated by means of 0.1 N H<sub>2</sub>SO<sub>4</sub> and 0.1 N NaOH solutions and measured using a multi-parameter analyser (CONSORT C831, Belgium). TSS, TDS, and VSS were analysed according to Standard Methods for the Examination of Water and Wastewater (APHA 2540C, 2540D, and 2540E). For TSS, a 25 mL sample was filtered through a pre-weighed glass fiber filter (Whatman GF/C, UK) using a vacuum filtration unit (Sartorius, Germany). The filter was then dried at 103–105 °C in a drying oven (Mettler, Germany) for 24 h, cooled in a desiccator, and reweighed to determine the mass

of suspended solids. For TDS, another 25 mL sample was evaporated at 180 °C in a drying oven (Mettler, Germany) until a constant weight was achieved. The residue was weighed using an analytical balance (Sartorius, Germany). For VSS, the TSS filter was placed in a muffle furnace (Nabertherm, Germany) at 550 °C for 30 min. After cooling in a desiccator, the filter was reweighed. The difference between TSS and the remaining mass after ignition represented the volatile suspended solids (VSS). The pH of CWW was regulated by means of 0.1 N H<sub>2</sub>SO<sub>4</sub> and 0.1 N NaOH solutions. All analyses were conducted in triplicate, and average values were reported in Table 3.

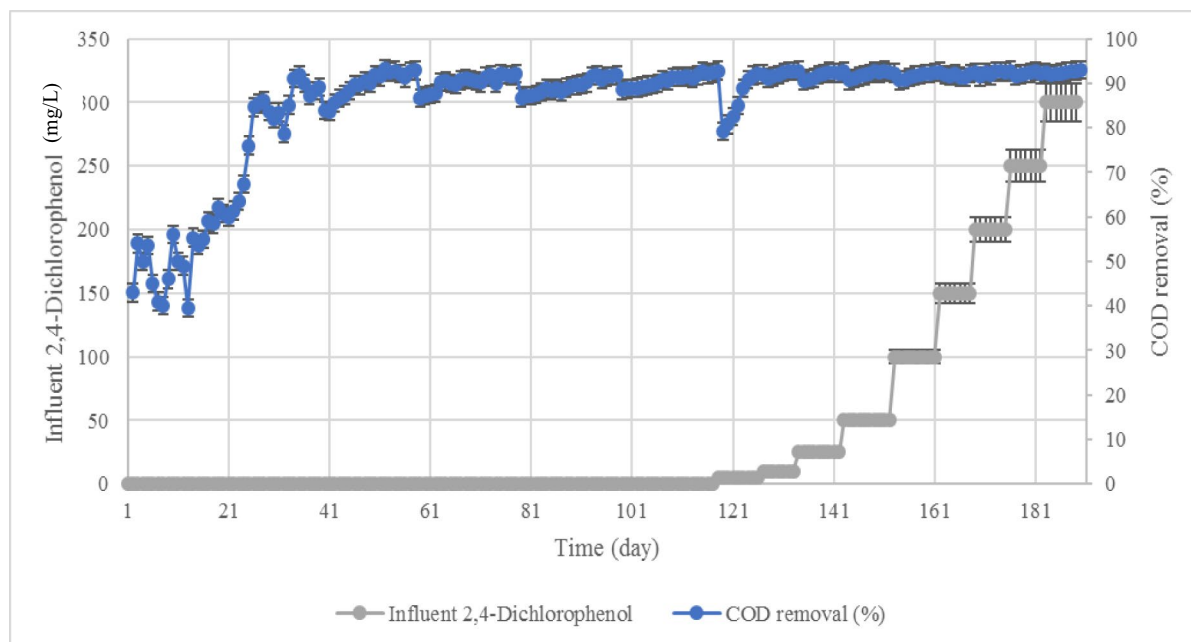
## 3 Results and Discussion

### 3.1 COD Removal During Anaerobic Membrane Bioreactor Operation

The COD removal performance of the anaerobic membrane bioreactor (AnMBR) is presented in Fig. 3. Initial fluctuations were observed in COD removal during the first 21 days of operation, likely due to microbial adaptation and system stabilization. As the COD concentration and organic loading rate increased, microbial acclimatization enhanced,

**Table 3** Specifications of concentration, organic loading and removal percentage COD and 2,4-DCP (mean ± SD) during reactor operation

| Operation days | Useful volume (L) | Influent COD | Influent 2,4-DCP | OLR (kg/m <sup>3</sup> .d) | Average COD removal (%) | Average 2,4-DCP removal (%) |
|----------------|-------------------|--------------|------------------|----------------------------|-------------------------|-----------------------------|
| 1–21           | 8                 | 1000         |                  | 0.125                      | 51.55 ± 7.2             |                             |
| 22–38          | 8                 | 2000         |                  | 0.250                      | 81.5 ± 9.3              |                             |
| 39–57          | 8                 | 3000         |                  | 0.375                      | 89.6 ± 2.98             |                             |
| 58–77          | 8                 | 4000         |                  | 0.50                       | 90.2 ± 1.7              |                             |
| 78–97          | 8                 | 5000         |                  | 0.625                      | 89.4 ± 1.7              |                             |
| 98–117         | 8                 | 6000         |                  | 0.750                      | 90.6 ± 1.3              |                             |
| 118–126        | 8                 | 6006.4       | 5                | 0.751                      | 91.6 ± 0.6              | 85.2 ± 4.9                  |
| 127–133        | 8                 | 6012.8       | 10               | 0.752                      | 92.1 ± 0.58             | 90.2 ± 4.4                  |
| 134–142        | 8                 | 6032         | 25               | 0.754                      | 91.9 ± 0.8              | 93.2 ± 2.1                  |
| 143–152        | 8                 | 6064         | 50               | 0.758                      | 92.04 ± 0.7             | 91.3 ± 6.6                  |
| 153–161        | 8                 | 6128         | 100              | 0.766                      | 91.7 ± 0.7              | 97.6 ± 1.6                  |
| 162–168        | 8                 | 6192         | 150              | 0.774                      | 91.9 ± 0.4              | 98.5 ± 1.4                  |
| 169–175        | 8                 | 6256         | 200              | 0.782                      | 92.3 ± 0.5              | 99.01 ± 0.8                 |
| 176–182        | 8                 | 6320         | 250              | 0.790                      | 92.3 ± 0.4              | 91.9 ± 2.7                  |
| 183–189        | 8                 | 6384         | 300              | 0.798                      | 92.5 ± 0.4              | 80.6 ± 4.4                  |



**Fig. 3** COD removal rate by bioreactor compared to 2,4-DCP concentration of incoming wastewater during operation

resulting in a steady improvement in COD removal efficiency, reaching over 80%. After 156 days of operation, the system achieved its maximum COD removal efficiency of 99.63%, indicating effective microbial adaptation and bioreactor performance. The progressive increase in COD removal aligns with previous studies, such as Nabavi et al. (Nabavi et al., 2013), who demonstrated high efficiency (99.8%) in treating COD using a sequencing batch biofilm reactor (SBBR) system. The high COD removal efficiency observed in this study also correlates with findings by De Jager et al. (De Jager et al., 2012), who reported that a dual-stage MBR achieved COD removal rates of 97% over a nine-month test period. Amin et al. (Amin et al., 2016) also obtained an average COD removal efficiency of 94.86% using an MBR system.

The average COD removal efficiency in this study was 85.93%, with a standard deviation of 12.8%. The maximum and minimum recorded removal rates were 93.17% and 39.5%, respectively. A notable decline in COD removal efficiency occurred between days 118 and 123, attributed to the injection of 2,4-dichlorophenol (2,4-DCP), which temporarily stressed the microbial community. However, following a short adaptation period, the system recovered, and removal efficiency returned to stable levels. These findings align with

previous research. Yang et al. (Yang et al., 2012) studied the application of submerged membrane bioreactors (MBRs) following electrocoagulation and electroflotation processes for treating simulated restaurant wastewater, which contained high levels of oil, grease, suspended solids, and detergents. The study reported overall chemical oxygen demand (COD) removal efficiencies of 98.3% and 99.1% for influent containing 5 mg/L and 100 mg/L of oil, respectively, emphasizing the significant role of biological treatment in MBR systems. Similarly, Bornare et al. (Bornare et al., 2015) evaluated the performance of a submerged AnMBR treating synthetic wastewater with an influent COD of 4,759 mg/L. The study reported a COD removal efficiency exceeding 95%, with treated effluent COD concentrations of 231 mg/L at a hydraulic retention time of 25.5 h. Another study by Chen et al. (Chen et al., 2017) investigated the performance of a submerged anaerobic membrane bioreactor (SAnMBR) in treating sewage. The system achieved average removal efficiencies of 90% for COD.

The COD removal performance observed in this study is also consistent with other anaerobic membrane bioreactor applications. For instance, Ji et al. (Ji et al., 2020) reported COD removal of ~89% using AnMBRs for municipal wastewater. Similarly,

Wu et al. (Wu et al., 2021) achieved ~90% COD removal while treating landfill leachate in a high-solids AnMBR system. Pang et al. (Pang et al., 2024) treated phenol- and quinoline-contaminated wastewater and recorded 95% COD removal, reinforcing the high efficiency of anaerobic membrane systems under complex wastewater conditions. These results confirm that MBR systems, particularly AnMBRs, offer a robust and highly efficient approach for COD removal in wastewater treatment.

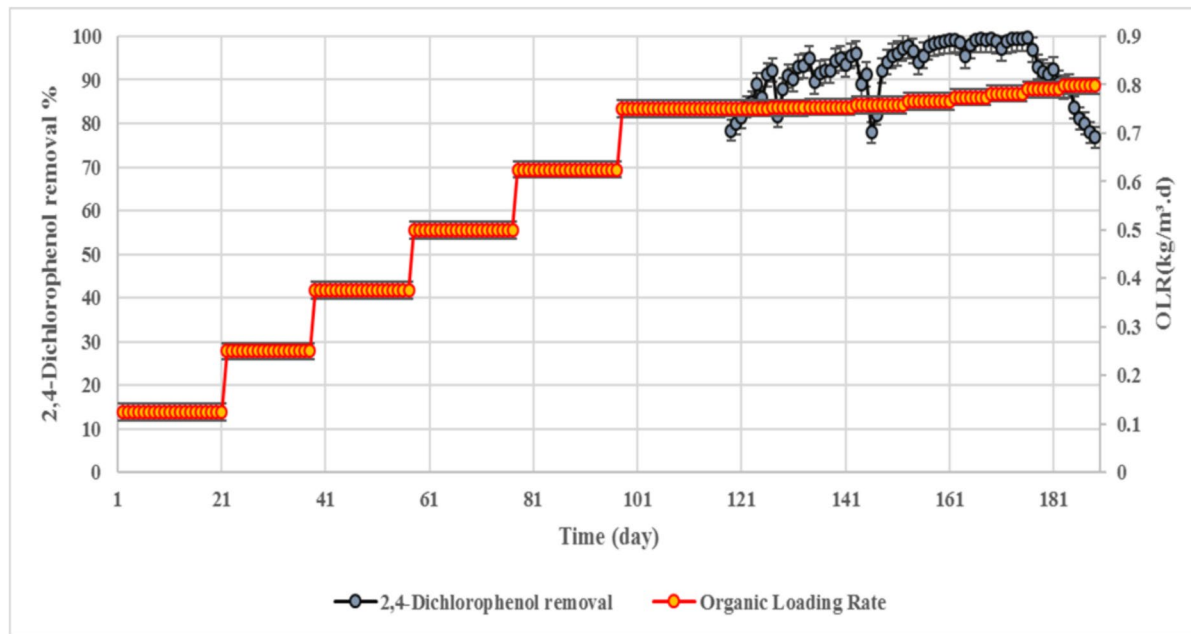
The initial lag phase in COD removal can be attributed to microbial acclimatization. During early reactor operation, syntrophic communities likely established cooperative metabolic pathways for breaking down complex organics. The gradual increase in COD removal suggests successful enrichment of fermentative and methanogenic consortia, which are essential in anaerobic environments. The stability observed post-adaptation reflects a balanced microbial ecosystem capable of handling variable organic loading (Zhu et al., 2023).

### 3.2 2,4-DCP Removal During SAnMBR Operation

Figure 4 illustrates the removal efficiency of 2,4-dichlorophenol (2,4-DCP) in relation to the

organic loading rate (OLR) over time. The average 2,4-DCP removal efficiency during the operation was 91.9%, with a minimum and maximum removal of 76% and 99.6%, respectively. As the 2,4-DCP concentration increased, the system maintained a high removal rate, indicating the robustness of the submerged anaerobic membrane bioreactor (SAnMBR) in treating phenolic compounds.

Previous studies have explored phenol and chlorophenol biodegradation using various bioreactor configurations. A study by Praveen and Loh (Praveen & Loh, 2013) developed a hollow fiber membrane bioreactor (HFMB) impregnated with trioctylphosphine oxide (TOPO) for the two-phase biodegradation of phenol using *Pseudomonas putida* ATCC 11172. The system effectively degraded phenol concentrations ranging from 800 to 2,500 mg/L, achieving complete degradation of 1,000 mg/L phenol within 12 h at a specific growth rate of  $0.73 \text{ h}^{-1}$ . The bioreactor maintained stable performance over 400 h under various operating conditions, demonstrating the stability of TOPO impregnation within the membrane. Similarly, a recent study by Rezakazemi et al. (Rezakazemi et al., 2018) investigated the treatment of phenolic wastewater using an anaerobic submerged membrane bioreactor



**Fig. 4** 2,4-DCP removal rate in bioreactor compared to organic load applied to bioreactor

(ASMBR). The researchers examined the impact of varying solids retention times and different carbon-to-nitrogen (C/N) ratios in synthetic wastewater. They increased the influent phenol concentration stepwise from 100 mg/L to 1000 mg/L over the first 30 days and maintained it at 1000 mg/L thereafter. With a chemical oxygen demand (COD) to nitrogen ratio of 100:5.0 during the initial 100 days, the system achieved phenol and COD removal efficiencies exceeding 99% and 95%, respectively. Adjusting the COD to nitrogen ratio to 100:2.1 in the latter phase restored complete ammonium removal. These findings suggest that maintaining an appropriate C/N ratio is crucial for optimizing phenol removal in ASMBR systems treating high-strength phenolic wastewater. The operational mode (continuous vs. intermittent feeding) plays a crucial role in biodegradation efficiency. Carbajo et al. (Carbajo et al., 2010) investigated phenol removal using an anaerobic fluidized bed reactor in the presence of volatile fatty acids as auxiliary substrates, reporting 95% efficiency after 212 days under continuous feeding. In contrast, with intermittent feeding, the same removal efficiency was achieved in just 32 days (24).

Based on previous studies, the presence of auxiliary substrates can enhance the biodegradation of recalcitrant compounds, provided that these substrates are highly biodegradable. However, excessive auxiliary substrates may have inhibitory effects. In this study, increasing the auxiliary substrate concentration up to 6000 mg/L for 2,4-DCP removal (up to 300 mg/L) did not exhibit any inhibitory effects on the SANMBR performance, further demonstrating its resilience in treating chlorophenolic compounds.

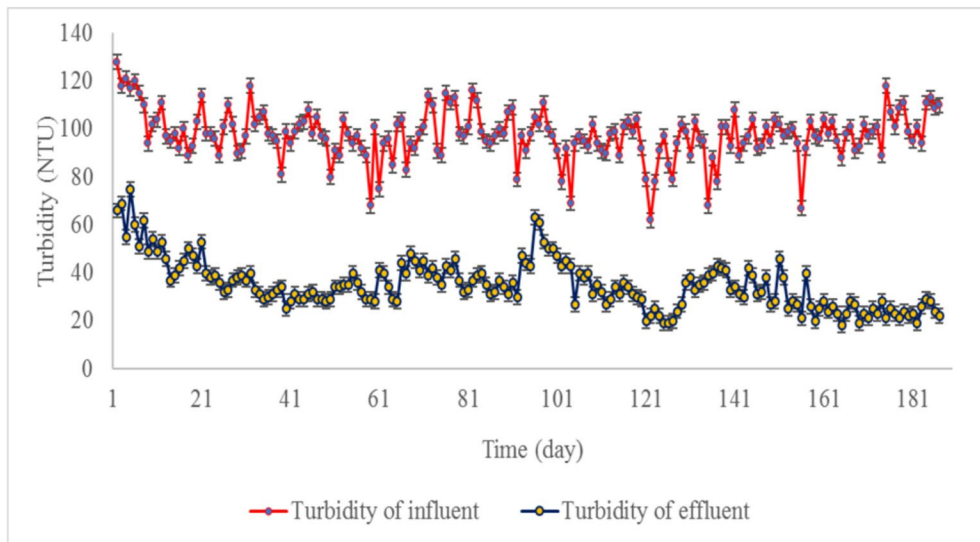
The findings of the present study are further supported by Almasi et al. (2018), who achieved 98.62% phenol removal using molasses as an auxiliary substrate in anaerobic systems. However, unlike their study, present system attained high 2,4-DCP removal without the addition of external carbon sources, indicating greater resilience. Moreover, Garcia Rea et al. (Garcia Rea et al., 2022) reported 100% removal of phenolic compounds under saline conditions, which highlights the robustness of anaerobic systems in toxic environments.

The system's ability to maintain high 2,4-DCP removal under increasing concentrations further

supports effective microbial adaptation. The microbial community likely underwent selective enrichment favoring chlorophenol-degrading anaerobes, possibly utilizing reductive dechlorination followed by ring cleavage (Azizi et al., 2021). These mechanisms are well documented in the degradation of halogenated phenols under anaerobic conditions. Additionally, the progressive improvement in 2,4-DCP removal efficiency over time suggests inducible enzymatic responses within the microbial consortia. Enzymes such as dehalogenases, phenol hydroxylase, and catechol dioxygenases may have played a role in catalysing initial breakdown reactions, especially during adaptation phases. While enzyme expression may be latent or suppressed in early exposure, prolonged contact with chlorinated phenols likely triggered the upregulation of detoxification and metabolic pathways. These shifts would enable microbes to transform 2,4-DCP into less toxic intermediates through dechlorination, followed by ring cleavage and conversion into short-chain fatty acids, ultimately supporting methanogenesis. Thus, the removal trend observed in this study can be attributed to both microbial acclimatization—marked by population shifts toward more tolerant or specialized taxa—and enzyme-driven biotransformation of 2,4-DCP. Together, these mechanisms illustrate the adaptive capacity of SANMBRs to handle refractory phenolic compounds under variable loading conditions.

### 3.3 Influent Wastewater and Effluent Wastewater Turbidity During Operation

Turbidity is a key aesthetic and physicochemical parameter of wastewater, primarily influenced by the concentration of suspended particles, microbial content, and organic matter. Figure 5 illustrates the turbidity variations of both influent and effluent wastewater throughout the bioreactor operation. The average turbidity of the influent wastewater was recorded at 97.74 NTU, with a median value of 98 NTU and a mode of 97 NTU. Since the influent was synthetically prepared using glucose and 2,4-DCP, its turbidity remained relatively low compared to real wastewater sources. Conversely, the effluent wastewater exhibited an average turbidity of 35.09 NTU, indicating a notable reduction in turbidity levels. The system achieved an overall turbidity removal efficiency of 63.98%. This reduction can be attributed to several



**Fig. 5** Turbidity of influent and effluent in comparison to each other

factors, including the effective retention of suspended solids by the submerged anaerobic membrane bioreactor (SAnMBR) and the biological degradation of organic compounds within the reactor. Similar findings have been reported in previous studies, where membrane bioreactor systems demonstrated high turbidity removal efficiencies, typically ranging between 60 and 90% (Asante-Sackey et al., 2022; Boano et al., 2020). The presence of a membrane filtration barrier significantly enhances solid–liquid separation, ensuring minimal suspended solids in the effluent (Othman et al., 2021).

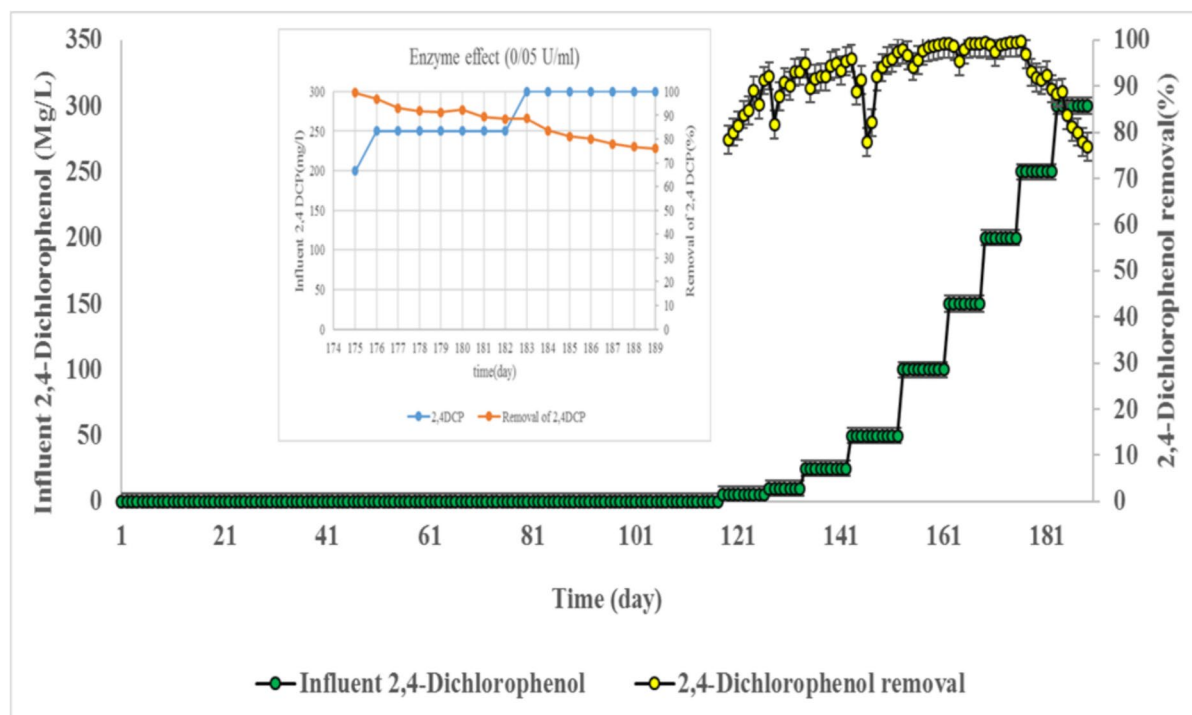
Comparable performance was noted by Gavlak et al. (Gavlak, Aguiar Battistelli et al. 2024), who reported turbidity removal efficiencies up to 99% using a SAnMBR treating plywood industry wastewater. Although turbidity reduction in the current study was slightly lower (63.98%), the difference may be attributed to the synthetic nature of our influent and lower initial particulate load.

The turbidity trends observed in the bioreactor can be explained by the interplay of several operational and physicochemical factors. The influent wastewater, composed of glucose and 2,4-DCP, contained limited complex particulates, leading to relatively stable turbidity levels. However, fluctuations in influent turbidity were likely due to variations in organic loading and microbial activity. The gradual decline in effluent turbidity over time suggests effective sludge retention

and biomass adaptation within the system, contributing to improved particulate removal (Zhang et al., 2015). Additionally, membrane fouling and periodic cleaning cycles may have influenced effluent turbidity. As filtration resistance increased due to biofouling and organic deposition, effluent quality may have slightly deteriorated before recovering after membrane maintenance procedures. This phenomenon has been documented in other submerged membrane systems, where operational strategies such as backflushing and chemical cleaning help maintain consistent turbidity removal (Sohn et al., 2021).

### 3.4 Impact of Enzymatic Activity on 2,4-DCP Degradation

As shown in Fig. 6, the influent 2,4-DCP concentration remained relatively stable throughout the monitoring period, fluctuating around 250–300 mg/L. However, the removal efficiency exhibited a prolonged lag phase (0–120 days), followed by a gradual increase, ultimately surpassing 90% after approximately 180 days. This trend indicates a progressive acclimation of the microbial consortia within the anaerobic bioreactor to 2,4-DCP degradation. The initial phase (0–120 days) exhibited minimal removal, likely due to microbial adaptation and the toxic effects of 2,4-DCP on anaerobic bacteria. Chlorophenols are known to inhibit microbial growth due to



**Fig. 6** Effect of enzymatic activity on the degradation of 2,4-DCP

their hydrophobicity and cellular toxicity, which disrupts enzymatic activities.

During this lag period, the microbial community likely underwent a shift in structure and function, gradually favoring species capable of degrading halogenated aromatic compounds. Anaerobic degradation of 2,4-DCP is typically initiated by reductive dechlorination, where chlorine atoms are removed to yield less toxic and more biodegradable intermediates such as phenol. This is followed by ring cleavage and conversion into short-chain fatty acids and methane. The steep increase in removal after day 120 reflects the successful enrichment of functional microbial guilds—particularly obligate anaerobes such as sulfate-reducing and methanogenic bacteria—that participate in syntrophic interactions. These consortia are known to exhibit greater tolerance to toxic substrates over time, likely due to upregulation of stress-response genes and enhanced detoxification pathways.

Similar lag phases have been observed in anaerobic degradation studies of chlorinated organic compounds. For example, Sponza (Sponza & Uluköy, 2008) reported significant removal efficiencies after an adaptation period in an upflow anaerobic sludge

blanket reactor treating 2,4-DCP. Beyond 120 days, the system exhibited a sharp increase in removal efficiency, suggesting that selective enrichment of 2,4-DCP-degrading microorganisms occurred. This phenomenon aligns with findings by Yu et al. (Yu et al., 2023), who demonstrated that advanced oxidation processes, such as the Fenton process catalyzed by nanoscale zero-valent iron supported by biochar, can effectively degrade 2,4-DCP, achieving a 99.38% degradation rate under optimal conditions.

Figure 6 illustrates the effect of enzymatic activity (0.05 U/ml) on the degradation of 2,4-DCP over a shorter time span. The influent concentration remained stable around 275 mg/L, while removal efficiency initially reached 85% but gradually declined to approximately 75% by day 189. This suggests that while enzyme-mediated degradation contributed to 2,4-DCP breakdown, long-term stability was not maintained, possibly due to enzyme deactivation or microbial community shifts.

It is likely that enzyme supplementation supported early-stage degradation via extracellular catalysis, particularly through pathways involving monooxygenases and dioxygenases. These include

enzymes such as phenol hydroxylase, which catalyzes hydroxylation of phenol to catechol, and catechol 2,3-dioxygenase, which cleaves the aromatic ring structure. However, in the anaerobic environment, the activity of such enzymes may be limited or short-lived due to instability or inhibition by accumulating intermediates. The decline in removal efficiency during continuous enzyme addition suggests that sustained removal relies more on adaptive microbial metabolism rather than exogenous enzymatic input.

Comparable results were reported by Chris et al. (Chris Felshia et al., 2020), where *Bacillus licheniformis* strain SL10 exhibited a 97% degradation efficiency of 2,4-DCP under optimal conditions. The degradation was facilitated by extracellular enzymes, including catechol 2,3-dioxygenase and phenol hydroxylase, indicating the significance of enzymatic activity in the breakdown of 2,4-DCP.

Several studies have evaluated the removal of phenolic compounds in anaerobic membrane bioreactors (AnMBRs). For instance, Sponza (Sponza & Uluköy, 2008) reported significant removal of phenolic pollutants in an AnMBR treating industrial wastewater, with a similar adaptation phase before reaching peak efficiency. Additionally, Yu et al. (Yu et al., 2023) demonstrated that combining advanced oxidation processes with anaerobic treatment improved overall process stability and ensured sustained removal of toxic compounds.

In comparison, the present study illustrates that microbial acclimatization under prolonged exposure can achieve comparable or superior removal efficiencies without the need for aggressive chemical oxidation. The observed trends suggest a gradual development of functional biodegradation capacity within the microbial community, supported by

internal enzymatic systems and resilient metabolic interactions. While SANMBRs offer high treatment efficiency, optimization strategies such as bioaugmentation, co-substrate addition, or in situ enzyme production could potentially reduce the adaptation period. Additionally, membrane fouling was identified as a key operational challenge in similar studies, which may have influenced the gradual improvement in removal efficiency observed in this study.

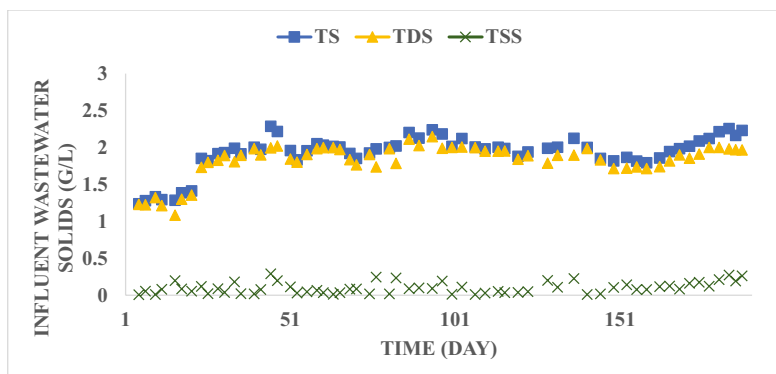
### 3.5 Trends in Suspended and Dissolved Solids

The performance of the anaerobic membrane bioreactor (AnMBR) in removing total solids (TS), total dissolved solids (TDS), and total suspended solids (TSS) was evaluated over an operational period of 180 days (Fig. 7). The influent concentrations of TS, TDS, and TSS exhibited fluctuations, with TS ranging from 1.0 to 2.5 g/L, TDS from 1.0 to 1.8 g/L, and TSS from 0.1 to 0.4 g/L.

The AnMBR demonstrated high removal efficiencies for these parameters. The TS removal efficiency ranged from 84 to 96%, indicating effective retention of both suspended and dissolved solids. Similarly, TDS removal efficiency ranged from 83 to 94%, reflecting the system's capability to manage dissolved constituents. TSS removal efficiency was consistently high, ranging from 85 to 95%, underscoring the membrane's effectiveness in retaining suspended solids.

These findings align with previous studies on AnMBR systems. For instance, Trzcinski and Stuckey (Trzcinski & Stuckey, 2009) reported that an AnMBR treating the organic fraction of municipal solid waste achieved a chemical oxygen demand (COD) removal efficiency greater than 90% at a hydraulic retention time (HRT) of 1.6–2.3 days, with soluble COD in the permeate ranging from 400 to 600 mg/L.

**Fig. 7** Influent concentrations of total solids (TS), total dissolved solids (TDS), and total suspended solids (TSS) over the operational period. The data reflects the stable performance of the AnMBR in retaining and reducing solid concentrations throughout the study period



**Table 4** Comparison of SAnMBR performance in this study with literature

| Reactor Type                     | Wastewater Type                      | COD Removal (%)         | Phenol/2,4-DCP Removal (%) | OLR (kg/m <sup>3</sup> ·d) | Notable Features                                 | Reference                                |
|----------------------------------|--------------------------------------|-------------------------|----------------------------|----------------------------|--------------------------------------------------|------------------------------------------|
| SAnMBR with hollow-fiber         | Synthetic with 2,4-DCP               | 99.63 (max), 91.9 (avg) | 99.6 (max), 91.9 (avg)     | 0.125–0.798                | Long-term operation, high 2,4-DCP removal        | This Study                               |
| SAnMBR                           | Synthetic wastewater                 | > 95                    | –                          | ~0.5                       | Influent COD ~4759 mg/L                          | (Bornare et al., 2015)                   |
| SAnMBR                           | Sewage                               | 90                      | –                          | NA                         | Stable biogas production                         | (Chen et al., 2017)                      |
| ASMBR                            | Synthetic with phenol + ammonium     | > 95                    | > 99                       | NA                         | Evaluated different C/N ratios                   | (Rezakazemi et al., 2018)                |
| Anaerobic Reactor                | Phenol + molasses                    | NA                      | 98.62                      | NA                         | Used auxiliary substrate (molasses)              | (Almasi et al., 2018)                    |
| SAnMBR                           | Plywood industry wastewater          | 98                      | 70                         | NA                         | High turbidity removal (99%)                     | (Gavlak, Aguiar Battistelli et al. 2024) |
| AnMBR                            | Saline wastewater (p-cresol, phenol) | –                       | 100                        | NA                         | Full phenol compound removal under saline stress | (Garcia Rea et al., 2022)                |
| AnMBR                            | Phenol + quinoline                   | 95                      | > 99                       | Higher than this study     | Evaluated polyaluminum chloride addition         | (Pang et al., 2024)                      |
| Flat sheet & Hollow-fiber AnMBRs | Seafood processing wastewater        | 79.5                    | NA                         | NA                         | Compared membrane types                          | (Phat, Thi Thai Hang et al. 2025)        |

Additionally, a study on the effect of TDS on the performance of a sequencing batch reactor (SBR) treating synthetic wastewater found that COD removal efficiencies of 94.8% and 92.2% were achieved at TDS levels of 750 and 1500 mg/L, respectively (Wu & Maskaly, 2018).

Tchobanoglous et al. (Tchobanoglous, Burton et al. 2002) noted that TSS concentrations in wastewater typically range from 0.1 to 1.0 g/L, with the lower values being typical of treated influents, such as in this study. The relatively low TSS values in the present study further support the hypothesis of efficient pre-treatment processes. The findings also suggest that the fraction of suspended solids entering the system has already been reduced prior to sampling, potentially through advanced pre-treatment technologies or operational optimizations. Similarly, Lawrence et al.

(Lawrence et al., 2024) found stable TSS concentrations with high removal efficiency (98–99%), despite seasonal variations in temperature and rainfall, and observed no significant impact on TSS removal.

The high removal efficiencies for TS, TDS, and TSS observed in this study reflect the enhanced performance of the hollow-fiber membrane-based AnMBR system compared to conventional membrane bioreactors. The improved TS and TDS removal efficiencies can be attributed to the high sludge retention time and effective separation capacity of the membrane, which facilitated enhanced microbial degradation and solid retention. The consistent TSS removal highlights the role of membrane filtration in maintaining low effluent turbidity and preventing particle breakthrough. The combination of biological treatment and membrane filtration in the AnMBR system

effectively stabilized solid concentrations and ensured long-term operational stability.

These observations are in agreement with findings by Phat et al. (Phat, Thi Thai Hang et al. 2025), who compared flat-sheet and hollow-fiber AnMBRs. Their study showed TSS removal around 98.9% and TDS removal below 11%. In contrast, the present study achieved TSS and TDS removal of 95% and 94% respectively, highlighting better performance for dissolved solids likely due to longer retention time and enhanced microbial adaptation. The findings from this study align with previous reports on AnMBR performance, confirming that the newly designed hollow-fiber membrane-based AnMBR system offers significant advantages in terms of solid retention and removal efficiency in wastewater treatment applications.

Although membrane fouling was not directly quantified in terms of transmembrane pressure or filtration resistance, operational observations suggested gradual fouling behavior, particularly during the later phases of the 180-day run. The presence of 2,4-DCP and elevated COD levels may have contributed to increased biofilm formation and cake layer accumulation on the membrane surface. Periodic declines in effluent clarity and recovery following cleaning cycles indicated reversible fouling effects. These behaviors are typical of submerged anaerobic membrane systems, where biofouling is driven by extracellular polymeric substances, microbial growth, and organic deposition. Strategies such as gas scouring and intermittent cleaning are essential for fouling control and were used as part of routine operation. These findings are consistent with prior reports on AnMBR fouling dynamics under sustained toxic and organic loads (Sohn et al., 2021; Wu et al., 2021).

A consolidated comparison of the SAnMBR performance in this study with prior research is summarized in Table 4 to highlight operational efficiency and contextualize removal performance across various systems.

## 4 Conclusions

This study demonstrated the successful long-term application of a submerged anaerobic membrane bioreactor (SAnMBR) equipped with hollow-fiber membranes for treating high-strength phenolic wastewater

containing 2,4-dichlorophenol (2,4-DCP). The system operated effectively for 189 days under mesophilic conditions ( $36 \pm 1$  °C), maintaining stable performance despite fluctuations in organic loading and influent toxicity.

Key findings include:

- Maximum COD removal efficiency of 99.63%, with an average of 91.9% sustained throughout the operation;
- Peak 2,4-DCP removal of 99.6%, with a mean removal efficiency of 91.9%, confirming the reactor's robustness in handling toxic phenolic compounds;
- TSS and TDS removal efficiencies of 95% and 94%, respectively, highlighting strong physical retention and biodegradation capabilities;
- Turbidity reduction by 63.98%, contributing to improved effluent quality.

The system exhibited a short adaptation period following 2,4-DCP introduction, after which microbial communities acclimated effectively, enabling high-level contaminant degradation. The hollow-fiber membrane facilitated efficient solid–liquid separation and minimized fouling, supporting consistent transmembrane pressure and prolonged membrane performance.

Overall, the results confirm the SAnMBR's potential as a reliable and sustainable solution for industrial wastewater treatment, especially for effluents containing recalcitrant phenolic pollutants. Future research should focus on enhancing microbial adaptation strategies, integrating fouling control techniques, and exploring scale-up potential for full-scale industrial applications.

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**Data Availability** The data used to support the findings of this study are included in the article. Should further data or information be required, these are available from the corresponding author upon request.

## Declarations

**Ethical Approval and Consent to Participate** This study does not involve animal or human subjects.

**Consent for Publication** All authors have consent to publish this paper with this journal.

**Competing Interests** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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